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PROCESS FOR THE PREPARATION OF (6S)- AND (6R)- TETRAHYDROFOLIC
ACID

We, EPROVA AKTIENGESELLSCHAFT, of Im Laternenacker 5, CH-8200 Schaffhausen, Switzerland, a company organized according to the laws of Switzerland do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

Process for the preparation of (6S)- and
(6R)-tetrahydrofolic acid

The invention relates to a process for the preparation of N-[4-[(2-amino-1,4,5,6,7,8-hexahydro-4-oxo-(6S)-pteridiny]methylamino]benzoyl]-L-glutamic acid (called (6S)-tetrahydrofolic acid in the following) and its salts and N-[4-[(2-amino-1,4,5,6,7,8-hexahydro-4-oxo-(6R)-pteridiny]methylamino]benzoyl]-L-glutamic acid (called (6R)-tetrahydrofolic acid in the following) and its salts.

Tetrahydrofolic acid derivatives contain 2 asymmetric centers. In this case, owing to the synthesis of these derivatives from folic acid, N-(pteroyl)-L-glutamic acid, the optically active C atom contained in the glutamic acid residue is present in the L-form, whereas the optically active C atom in position 6 formed by hydrogenation of the double bond in the 5,6-position of the pteroyl radical is in the racemic, (6R,S)-form. Synthetic derivatives of tetrahydrofolic acid therefore consist of a 1:1 mixture of 2 diastereomers. On natural occurrence, for example in the liver, the tetrahydrofolates are found only in one diastereomeric form, 5,6,7,8-tetrahydrofolic acid being in the (6S)-form.

As medicaments, tetrahydrofolates are mainly used as the calcium salt of 5-formyl-5,6,7,8-tetrahydrofolic acid (leucovorin) or 5-methyl-5,6,7,8-tetrahydrofolic acid for the treatment of megaloblastic folic acid anaemia, as an antidote for increasing the tolerability of folic acid antagonists, especially of aminopterin and methotrexate in cancer therapy ("leucovorin rescue"), for increasing the therapeutic effect of 5-fluorouracil and for the treatment of autoimmune diseases such as psoriasis and rheumatoid arthritis and for increasing the tolerability of certain antiparasitics, for example trimethoprim-sulfamethoxazole, in chemotherapy.

Tetrahydrofolic acid is used as the basic substance for the preparation of diverse tetrahydrofolic acid derivatives.

Efforts to prepare (6S)- or (6R)-tetrahydrofolic acid have hitherto led to the following methods:

- enzymatic methods
- physicochemical methods
- chemical methods

Enzymatic methods comprise reduction, normally carried out chemically, of folic acid to 7,8-dihydrofolic acid and subsequent enzymatic reduction thereof to (6S)-5,6,7,8-tetrahydrofolic acid, for example according to L. Rees et al., Tetrahedron 42(1), 117-36 (1986) or EP-A2-0,356,934. However, these processes can only be stopped with difficulty in the chemical step at the 7,8-dihydrofolic acid stage and also typically give only very small space-time yields in the enzymatic step, require expensive co-factors such as NADPH and necessitate a usually complex working-up methodology. Methods for the enzymatic preparation of optically pure tetrahydrofolic acid known hitherto are not suitable for the preparation of this compound on the industrial scale.

The separation of the diastereomer pairs was also attempted by means of chromatography, J. Feeney et al., Biochemistry, 20, 1837, (1981). These methods are not suitable for the preparation of the diastereomers on the industrial scale.

An asymmetric reduction of folic acid on chiral electrodes is also known from the group of physicochemical processes, S. Kwee et al., Bioelectrochem. Bioenerg. 7, 693-698, (1980). Owing to the concentrations of folic acid (typically 10^{-3} M) permitted during the reduction and the removal of the asymmetric inductor, which can only be carried out with difficulty, after reduction has taken place, these reactions, however, cannot be employed for industrial preparation.

From the field of chemical synthesis, the possibility of asymmetric hydrogenation of folic acid in the presence of an optically active catalyst exists, for example according to P.H. Boyle, et al., J. Chem. Soc. Chem. Commun. (1974), 10, 375-6. However, this requires the use of very expensive catalysts, which, after

homogeneous catalysis has taken place, can only be separated off with great wastage of the product.

There is to date therefore still no industrially utilizable process for obtaining optically pure tetrahydrofolic acid.

The object was thus to work out a simple, industrially utilizable and economical method for the preparation of optically pure tetrahydrofolic acid.

It has now surprisingly been found that correspondingly optically enriched tetrahydrofolic acid addition salt is precipitated from aqueous solutions of (6R,S)-tetrahydrofolic acid or its salts after addition of sulfonic acids or sulfuric acid. This can be removed by filtration. The diastereomeric addition salt can be isolated from the filtrate. Both salts can then be purified both chemically and optically by recrystallization and/or liberation of tetrahydrofolic acid and subsequent conversion to the salt. It is all the more surprising that optical resolution takes place during the crystallization of the sulfonic acid or sulfuric acid addition salt as no optical enrichment can be detected by preparation/recrystallization of other salts, for example the hydrochloric acid addition salt (W. Frick, et al., *Helv. Chim. Acta*, 57, 2658-61 (1974)). No enrichment of one of the epimeric forms of tetrahydrofolic acid can be achieved even with other strong acids such as hydrobromic acid, hydriodic acid, nitric acid, phosphoric acid, formic acid, oxalic acid, chloro-, dichloro- and trichloroacetic acid.

The invention relates to a process for the preparation of (6S)- and (6R)-tetrahydrofolic acid and of their addition salts with sulfonic acids or with sulfuric acid, characterized in that (6R,S)-tetrahydrofolic acid is reacted with a sulfonic acid or with sulfuric acid, resulting acid addition salt is fractionally crystallized and, if desired, the (6S)- and/or (6R)-tetrahydrofolic acid is liberated from the resulting diastereomeric acid addition salts by treatment with a base and isolated.

The (6R,S)-tetrahydrofolic acid used here can be

employed either as the isolated product or alternatively preferably directly in situ as the product of reduction of folic acid.

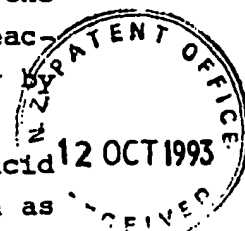
5 Sulfonic acids suitable for the process according to the invention are aromatic sulfonic acids having 6 to 14 C atoms, araliphatic sulfonic acids having 7 to 9 C atoms or aliphatic sulfonic acids having 1 to 3 C atoms. Suitable aromatic sulfonic acids are mainly benzenesulfonic acid, toluenesulfonic acids, xylenesulfonic acids, 10 nitrobenzenesulfonic acids, chlorobenzenesulfonic acids, nitrotoluenesulfonic acids, naphthalenesulfonic acids, substituted naphthalenesulfonic acids, naphthalenedisulfonic acids, camphorsulfonic acids, benzimidazole-sulfonic acids, substituted benzimidazolesulfonic acids, 15 such as, for example, 2-phenylbenzimidazole-5-sulfonic acid and many others.

20 A suitable araliphatic sulfonic acid is mainly phenylmethanesulfonic acid, and suitable aliphatic sulfonic acids are mainly methanesulfonic acid and ethanesulfonic acid.

Preferred addition salts for the process according to the invention are the benzenesulfonic acid, a toluenesulfonic acid and the sulfuric acid addition salts.

25 The crystallization is carried out from a polar medium. A suitable medium is in particular water or a mixture of water and lower (C_1 - C_6)-aliphatic water-soluble carboxylic acids, in particular acetic acid and lactic acid or liquid water-soluble amides such as formamide, 30 dimethylformamide, dimethylacetamide, 1-methylpyrrolidone and 2-piperidinone. The mixture customarily contains at least 50 % of water. The use of such a mixture normally increases the optical purity of the products, but the yield can decrease. Depending on the desired aim and the 35 particular starting material, the optimum for the reaction conditions can be determined without difficulty by systematic tests.

Owing to the sensitivity of tetrahydrofolic acid to oxidation, the use of an oxidation inhibitor such as



2-mercaptoethanol is recommended.

During the crystallization the acid addition salt of (6S)-tetrahydrofolic acid as a rule precipitates first - the diastereomeric (6R)-compound is enriched in the filtrates.

The optically enriched tetrahydrofolic acid can be very easily liberated again from the salts obtained by addition of a base.

A further possibility of chemical and optical purification is offered by the recrystallization of (6S)- or (6R)-tetrahydrofolic acid acid addition salts and/or the conversion to a salt with a sulfonic acid and/or sulfuric acid carried out subsequently to the liberation of the tetrahydrofolic acid.

As a result of this process, (6S)- and (6R)-tetrahydrofolic acid and their salts with strong bases or acids have become accessible very easily and particularly economically.

The invention also relates to the use of the (6S)- and (6R)-tetrahydrofolic acid and their addition salts with sulfonic acids or with sulfuric acids obtained according to the process for the preparation of optically pure 5,10-methylenetetrahydrofolic acids and their salts with strong bases or acids by treatment with formaldehyde. It is to be taken into consideration here that 5,10-methylene-(6R)-tetrahydrofolic acid is obtained from (6S)-tetrahydrofolic acid by reaction with formaldehyde and 5,10-methylene-(6S)-tetrahydrofolic acid is obtained analogously from (6R)-tetrahydrofolic acid.

Examples to illustrate the invention:

The following HPLC method was employed to determine the purity of tetrahydrofolic acid, 5-formyl-, 5-methyl- and 5,10-methylenetetrahydrofolic acid:

Eluent A: 0.03 M Na_2HPO_4 + 0.03 M KH_2PO_4 in water

Eluent B: 1 part of (0.03 M Na_2HPO_4 + 0.03 M KH_2PO_4 in water)

3 parts of methanol
then adjusted to pH 7.8 with phosphoric acid

Gradient: from 2 % eluent B to 95 % eluent B in the course of 25 minutes

Column: ODS (Hypersil)

Detection: UV-300 nm

The following HPLC method was employed to determine the (6S)-content:

Derivatization: Dissolve tetrahydrofolic acid or addition salt in acetonitrile/water 1:1 and react with 2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl isothiocyanate

Eluent: 2.5 parts of acetonitrile
1.5 parts of methanol
6.0 parts of 0.02 M citric acid

Column: RP-8 (Lichrosphere)

Detection: UV-270 nm

Example 1

14.3 g of toluene-4-sulfonic acid (150 mol%) are dissolved at 60°C under nitrogen in 440 ml of water containing 0.1 % of 2-mercaptoethanol. 25.0 g of pure (6R,S)-tetrahydrofolic acid are introduced in the course of 5 minutes. The resulting suspension is cooled to 40°C. The precipitated product is filtered off after 2 - 5 hours, washed with water and then with ethanol.

16.9 g of toluene-4-sulfonic acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content of 86.7 % are obtained; determined by means of HPLC.

By recrystallization of the resulting product from a mixture of 110 ml of N,N'-dimethylformamide and

220 ml of water, toluene-4-sulfonic acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content of 97.5 % are obtained; determined by means of HPLC.

$[\alpha]_D^{25} = -60.6^\circ$ (c = 0.5 % in DMF)

To liberate the (6S)-tetrahydrofolic acid, 170 ml of water containing 0.1 % of 2-mercaptoethanol are cooled to 10°C under nitrogen. 5.0 g of toluene-4-sulfonic acid addition salt of (6S)-tetrahydrofolic acid are sprinkled in. 4 ml of 2 N sodium hydroxide solution are added dropwise to this suspension. After cooling to 2°C, the precipitated product is filtered off, washed with water and then with ethanol.

3.7 g of (6S)-tetrahydrofolic acid having a (6S)-content of 97.7 % are obtained; determined by means of HPLC.

$[\alpha]_D^{25} = -44.5^\circ$ (c = 1 % in water)

Example 2

13 g of toluene-4-sulfonic acid (135 mol%) are dissolved at 20°C under nitrogen in 200 ml of acetic acid and 200 ml of water containing 0.2 % of 2-mercaptoethanol. 25.0 g of pure (6R,S)-tetrahydrofolic acid are rapidly introduced. The solution is seeded with a little authentic (6S)-tetrahydrofolic acid-toluene-4-sulfonic acid addition salt. After 5 hours, the precipitated product is filtered off, washed with acetic acid/water and then with ethanol.

12.7 g of toluene-4-sulfonic acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content of 93.6 % are obtained; determined by means of HPLC.

10.0 g of the (6S)-tetrahydrofolic acid-toluene-4-sulfonic acid addition salt thus obtained are suspended at 25°C under nitrogen in 100 ml of water and adjusted to pH > 3.5 with 30 % sodium hydroxide solution. The pH of the solution thus obtained is then brought to below 1 again with 37 % hydrochloric acid. After 12 hours, the precipitated product is filtered off, washed with water and then with ethanol.

8.9 g of toluene-4-sulfonic acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content of

99.7 % are obtained; determined by means of HPLC.

$[\alpha]_D^{25} = -62.0^\circ$ ($c = 0.5$ % in DMF)

Example 3

14.30 g of toluene-4-sulfonic acid (150 mol%) are dissolved at 27°C under nitrogen in 220 ml of L(+)-lactic acid and 220 ml of water containing 0.2 % of mercaptoethanol. 25.0 g of pure (6R,S)-tetrahydrofolic acid are rapidly added. The resulting solution is seeded with a little authentic (6S)-tetrahydrofolic acid-toluene-4-sulfonic acid addition salt and cooled to 20°C. After 15 - 20 hours, the precipitated product is filtered off, washed with lactic acid/water and then with ethanol.

15.1 g of toluene-4-sulfonic acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content of 92.5 % are obtained; determined by means of HPLC.

Example 4

14.3 g of toluene-4-sulfonic acid (150 mol%) are dissolved at 27°C under nitrogen in 110 ml of 1-methyl-2-pyrrolidone and 110 ml of water containing 0.4 % of 2-mercaptoethanol. 25.0 g of pure (6R,S)-tetrahydrofolic acid are rapidly added. The solution is diluted with 220 ml of water and cooled to 20°C. After 15 - 20 hours, the precipitated product is filtered off, washed with 1-methyl-2-pyrrolidone/water and then with ethanol.

13.3 g of toluene-4-sulfonic acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content of 94.7 % are obtained; determined by means of HPLC.

Example 5

11.5 g of toluene-4-sulfonic acid (150 mol%) are dissolved at 27°C under nitrogen in 90 ml of N,N'-dimethylformamide and 90 ml of water containing 0.4 % of 2-mercaptoethanol. 20 g of pure (6R,S)-tetrahydrofolic acid are rapidly added. The solution is diluted with 180 ml of water and cooled to 20°C. After 15 - 20 hours, the precipitated product is filtered off, washed with N,N'-dimethylformamide/water and then with ethanol.

11.3 g of toluene-4-sulfonic acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content of 91.4 % are obtained; determined by means of HPLC.

Example 6

11.5 g of toluene-4-sulfonic acid (150 mol%) are dissolved at 27°C under nitrogen in 100 ml of N,N'-dimethylacetamide and 80 ml of water containing 0.4 % of 2-mercaptoethanol. 20 g of pure (6R,S)-tetrahydrofolic acid are rapidly added. The solution is diluted with 160 ml of water and cooled to 20°C. After 15 - 20 hours, the precipitated product is filtered off, washed with N,N'-dimethylacetamide/water and then with ethanol.

11.0 g of toluene-4-sulfonic acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content of 91.5 % are obtained; determined by means of HPLC.

Example 7

12 g of benzenesulfonic acid (150 mol%) are dissolved at 70°C under nitrogen in 440 ml of water containing 0.1 % of 2-mercaptoethanol. 25.0 g of pure (6R,S)-tetrahydrofolic acid are introduced in the course of 5 minutes. The resulting suspension is cooled to 60°C. After 2 - 5 hours, the precipitated product is filtered off, washed with water and then with ethanol.

13.8 g of benzenesulfonic acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content of 92.4 % are obtained; determined by means of HPLC.

10.0 g of the (6S)-tetrahydrofolic acid-benzenesulfonic acid addition salt thus obtained are suspended at 25°C under nitrogen in 100 ml of water and adjusted to pH > 3.5 with 30 % sodium hydroxide solution. The pH of the solution thus obtained is then brought below 1 again with 37 % hydrochloric acid. After 12 hours, the precipitated product is filtered off, washed with water and then with ethanol.

9.0 g of benzenesulfonic acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content of 99.8 % are obtained; determined by means of HPLC.

$[\alpha]_D^{25} = -63.5^\circ$ (c = 1 % in DMF)

If the 150 mol% benzenesulfonic acid employed is replaced by 55 mol% benzenesulfonic acid and 50 mol% hydrochloric acid, 12.7 g of benzenesulfonic acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content

of 91.6 % are obtained under identical crystallization conditions; determined by means of HPLC.

To liberate the 6R-tetrahydrofolic acid, the filtrate is adjusted to pH 3.5 with sodium hydroxide solution. After cooling to 5°C, the precipitated product is filtered off, washed with water and then with ethanol.

10 g of the residue thus obtained are dissolved at 50°C under nitrogen in 150 ml of water containing 0.1 % of 2-mercaptoethanol and 39 ml of 2 N sulfuric acid. After slowly cooling to 20°C over 15 hours and subsequently allowing to stand for 12 hours, the precipitated product is filtered off, washed with water and then with ethanol.

9.7 g of sulfuric acid addition salt of (6R)-tetrahydrofolic acid having a (6R)-content of 97.7 % are obtained; determined by means of HPLC.

Example 8

30 ml of 2M sulfuric acid are initially introduced at 60°C with 130 ml of water containing 0.2 % of 2-mercaptoethanol and 164 ml of glacial acetic acid. 20 g of pure (6R,S)-tetrahydrofolic acid are introduced in the course of 5 minutes. The resulting solution is cooled to 50°C. After 1 hour, the precipitated product is filtered off, washed with water/glacial acetic acid and then with ethanol.

11.0 g of sulfuric acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content of 65.5 % are obtained; determined by means of HPLC.

By recrystallizing 10 g of sulfuric acid addition salt of (6S)-tetrahydrofolic acid twice from dimethylformamide/water 1:3, 3.9 g of sulfuric acid addition salt of (6S)-tetrahydrofolic acid having a (6S)-content of 94.3 % are obtained; determined by means of HPLC.

Example 9

According to processes described in the literature, for example R.L. Blakley et al. Folates and Pterins, 1, 93-104 (1984) (6R,S)-tetrahydrofolic acid prepared in situ is directly reacted further with

32.2 g of benzenesulfonic acid addition salt of (6S)-tetrahydrofolic acid having a purity of 80 % and a (6S)-content of 94.2 % are obtained; determined by means of HPLC.

5 Example 11

By the replacement of toluene-4-sulfonic acid in Example 9 by the equivalent amount of sulfuric acid, the sulfate of (6S)-tetrahydrofolic acid can also be prepared in a similar manner.

10 27.1 g of (6S)-tetrahydrofolic acid sulfate having a purity of 85 % and a (6S)-content of 69.2 % are obtained; determined by means of HPLC.

Examples 12 - 18

15 The following can be prepared in a similar manner to that described in Examples 1 - 8:

12. Methanesulfonic acid addition salt of (6S)-tetrahydrofolic acid.
13. Ethanesulfonic acid addition salt of (6S)-tetrahydrofolic acid.
- 20 14. Phenylmethanesulfonic acid addition salt of (6S)-tetrahydrofolic acid.
15. Camphor-10-sulfonic acid addition salt of (6S)-tetrahydrofolic acid.
- 25 16. Naphthalene-1-sulfonic acid addition salt of (6S)-tetrahydrofolic acid.
17. Naphthalene-2-sulfonic acid addition salt of (6S)-tetrahydrofolic acid.
18. Naphthalene-1,5-disulfonic acid addition salt of (6S)-tetrahydrofolic acid.

30 Example 19

50 g of sulfuric acid addition salt of (6S)-tetrahydrofolic acid obtained according to Example 8 are dissolved with 200 ml of 2 N sodium hydroxide solution at 20°C under nitrogen in 500 ml of water. After addition of 7.5 ml of 36 % formaldehyde (125 mol%), a mixture of 275 ml of glacial acetic acid and 275 ml of 2 N sulfuric acid are added to the solution. After cooling to 2°C, the precipitated product is filtered off and washed through with ethanol.

39.6 g of 5,10-methylene-(6R)-tetrahydrofolic acid having a purity of 98.6 % and a (6R)-content of 99.6 % are obtained; determined by means of HPLC.

Example 20

28 g of benzenesulfonic acid addition salt of (6S)-tetrahydrofolic acid obtained according to Example 10 are dissolved with 30 % sodium hydroxide solution at about 25°C under nitrogen in 130 ml of water. After the addition of 44 ml of 36 % formic acid, the solution is divided and one half is treated with 3 g of NaBH₄. After 12 hours, it is acidified by addition of 10 ml of 37 % hydrochloric acid. The precipitated product is filtered off and washed through with water and ethanol.

11 g of 5-methyl-(6S)-tetrahydrofolic acid having a purity of 95.8 % and a (6S)-content of 99.5 % are obtained; determined by means of HPLC.

The other half is treated with excess calcium chloride, and the product which precipitates is filtered off and washed through with water and ethanol.

14 g of 5-formyl-(6S)-tetrahydrofolic acid calcium salt having a purity of 96.2 % and a (6S)-content of 99.7 % are obtained; determined by means of HPLC.

WHAT WE CLAIM IS:

1. Process for the preparation of (6S)- and (6R)-tetrahydrofolic acid and of their addition salts with sulfonic acids or with sulfuric acid, characterized in that (6R,S)-tetrahydrofolic acid is reacted with a sulfonic acid or with sulfuric acid, the resulting acid addition salt is fractionally crystallized and, if desired, the (6S)- and/or (6R)-tetrahydrofolic acid is liberated from the resulting diastereomeric acid addition salts by treatment with a base and isolated.

2. Process according to claim 1, wherein the crystallization is carried out from a polar medium.

3. Process according to claim 2, wherein the crystallization is carried out from water or a mixture of water with a C₁₋₆ aliphatic water-soluble carboxylic acid.

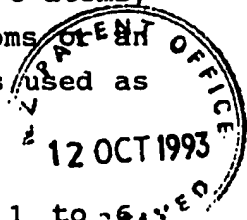
4. Process according to claim 3, wherein the carboxylic acid is acetic acid or lactic acid.

5. Process according to claim 2, wherein the crystallization is carried out from a mixture of water with a liquid water-soluble amide.

6. Process according to claim 5, wherein the amide is methylpyrrolidone, formamide, dimethylformamide or dimethylacetamide.

7. Process according to any one of preceding claims, wherein an aromatic sulfonic acid having 6 to 14 C atoms, an araliphatic sulfonic acid having 7 to 9 C atoms, or an aliphatic sulfonic acid having 1 to 3 C atoms is used as the sulfonic acid.

8. Process according to any one of claims 1 to 6, wherein the benzenesulfonic acid, toluene-4-sulfonic acid or sulfuric acid addition salt is used as addition salt.



9. (6S)-Tetrahydrofolic acid benzenesulfonate and (6R)-tetrahydrofolic acid benzenesulfonate.

10. (6S)-Tetrahydrofolic acid toluene-4-sulfonate and (6R)-tetrahydrofolic acid toluene-4-sulfonate.

11. (6S)-Tetrahydrofolic acid sulfate and (6R)-tetrahydrofolic acid sulfate.

12. Methanesulfonic acid, ethanesulfonic acid, phenylmethanesulfonic acid, camphor-10-sulfonic acid, naphthalene-1-sulfonic acid, naphthalene-2-sulfonic acid and naphthalene-1,5-disulfonic acid addition salts of (6S)-tetrahydrofolic acid.

13. Use of (6S)- or (6R)-tetrahydrofolic acid or of their addition salts with a sulfonic acid or with sulfuric acid, prepared according to any one of claims 1 to 8, as starting material for the preparation of optically pure 5,10-methylenetetrahydrofolic acid, 5-methyltetrahydrofolic acid or 5-formyltetrahydrofolic acid and their salts with strong bases or acids.

14. Use of salts of (6S)- or (6R)-tetrahydrofolic acid with sulfuric acid or a sulfonic acid as constituent of a medicament or as starting material for the preparation of a medicament.

15. A process according to claim 1 substantially as herein described or exemplified.

EPROVA AKTIENGESELLSCHAFT

By Their Attorneys

HENRY HUGHES LTD

Per:



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